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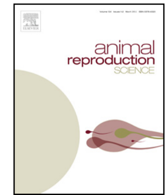

Effect of supplemental trace mineral level and form on peripubertal bulls☆

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ABSTRACT

Objectives were to determine if supplemental trace mineral levels and/or forms (sulfate and metal amino acid complexes) influence age at puberty, semen quality, endocrine status, and scrotal circumference in peripubertal bulls. Fifty peripubertal bulls were blocked by age and scrotal circumference and assigned to one of five treatments: (1) 1x sulfate form (1S); (2) 1x complexed form (1C); (3) 1S + 1C (2SC); (4) 1S + 2 × 1C (3SCC); and (5) 3 × 1S (3S). Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co. Liver biopsies were collected on d −21 and 100, and scrotal circumference, semen, and blood samples were collected on d −14, 14, 42, 70, and 98. All bulls were deficient in Cu yet adequate in Zn on d −21. Following 100 d on treatment, liver Zn concentrations decreased ($P < 0.01$) and liver Cu concentrations increased ($P < 0.01$) in bulls regardless of treatment. Day 100 liver Zn concentrations were similar ($P = 0.50$) across treatments, but liver Cu concentrations were greater ($P = 0.07$) in 3SCC and 3S bulls compared to 1C and 1S bulls, whereas 2SC bulls were intermediate. Bulls fed complexed minerals tended to reach puberty after fewer ($P = 0.11$) days on treatment (43.9 ± 5.7 d) than bulls fed only sulfate minerals (58.5 ± 6.7 d). Supplementing complexed Cu and Zn to prepubertal bulls may lower the age at puberty, however, no differences ($P \geq 0.40$) in semen characteristics or scrotal measurements ($P \geq 0.11$) were observed.

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1. Introduction

Producers use yearling bulls as a vital tool to accelerate genetic turnover of economically important traits. In addition, economic analyses of accumulated bull ownership and breeding costs associated with calf production per cow exposed, revealed greater profitability by using yearling bulls over 2-yr old bulls, given comparable genetic merit (Kasari et al., 1996).

In a review of 1276 Breeding Soundness Exam (BSE) records, 43% of bulls less than 15 months old were clas-

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sified as unsatisfactory breeders or classification deferred (Carson and Wenzel 1997). Elmore et al. (1975) re-evaluated 45 bulls less than two years of age that scored questionable or unsatisfactory on an initial BSE and reported 69% of these bulls' scores improved to satisfactory status 75 d later. This study concluded that the primary cause of these yearling bulls' failure to pass the initial BSE was due to immaturity.

Many of the factors that influence age of puberty in bulls are under direct control of management. Both Zn and Cu are involved in sexual maturity and reproductive development and maintenance of male ruminants (Hidiroglou, 1979). Assessing the correct Zn and Cu requirements for bull calves is important both from a production and economic standpoint. The current National Research Council (NRC, 2000) guidelines do not make adjustments in mineral requirements for cattle based on growth potential, levels of productivity, physiological status, stress levels, breed, or sex.

Due to the importance of Zn in male reproduction and the synergistic relationship of Cu with Zn, we felt that further investigation into the role of different levels and forms of trace minerals may be beneficial to producers. The objectives of this study were to determine if form and/or level of supplemental trace minerals fed to peripubertal bull calves influenced: (1.) liver trace mineral storage (2.) rate of sexual maturity (3.) quantity and quality of semen production and (4.) testicular development.

2. Materials and methods

2.1. Experimental design

This experiment was conducted at the USDA-ARS Livestock and Range Research Laboratory, Fort Keogh, in Miles City, MT. All procedures were approved by the Montana State University and Fort Keogh IACUC (IACUC No. 092702-1). Fifty crossbred bull calves, sired by one of four genetically similar Hereford sires, with an average initial body weight of 248 ± 31.5 kg were utilized. All bull calves had the same grandsire. Eighty-one days before initiation of the trial (d 0), all mineral supplements (free choice inorganic mineral) were removed from the bull calves and their dams. Bulls were weaned 47 d before the start of trial and were housed in feedlot pens to adjust to the basal diet. No mineral supplement was provided during this adjustment period. Bulls were allotted by puberty status, age (258 ± 8.9 d), and scrotal circumference (26.88 ± 2.3 cm) and assigned to one of five groups, of ten bulls each, to evaluate different trace mineral supplementation treatments: 1) 360 mg Zn, 200 mg Mn, 125 mg Cu, 12 mg Co in sulfate form (1S), 2) 360 mg Zn, 200 mg Mn, 125 mg Cu, 12 mg Co in complexed form (1C; Availa-4, Zinpro Corporation, Eden Prairie, MN), 3) 360 mg Zn, 200 mg Mn, 125 mg Cu, 12 mg Co in sulfate form plus 360 mg Zn, 200 mg Mn, 125 mg Cu, 12 mg Co in complexed form (2SC), 4) 360 mg Zn, 200 mg Mn, 125 mg Cu, 12 mg Co in sulfate form plus 720 mg Zn, 400 mg Mn, 250 mg Cu, 24 mg Co in complexed form (3SCC), and 5) 1080 mg Zn, 600 mg Mn, 375 mg Cu, 36 mg Co in sulfate form (3S).

Table 1
Nutrient composition of basal ration fed to bulls. Diet was formulated to provide a 1.2 kg per day gain. Diet contained 7% alfalfa hay, 10.5% corn, 75.5% corn silage, and 7% protein supplement.

DM, %	48.70
CP, %	13.70
NE_g, Mcal/kg	0.50
NE_m, Mcal/kg	0.70
TDN, %	66.60

Table 2
Trace mineral content (ppm) provided to bulls in 13.6 kg of basal diet plus respective supplement fed to peripubertal bulls.

	Zn	Cu	Mn	Co
Basal diet				
Alfalfa hay	1.3	0.5	1.5	<0.5
Corn grain	2.5	0.4	3.2	<0.5
Corn silage	3.3	3.3	24.3	<0.5
Total	7.1	4.2	29.0	<0.5
Treatment[a]				
1S	53	16	54	0.9
1C	53	16	52	1.1
2SC	76	22	56	1.5
3SCC	118	35	58	2.8
3S	115	32	74	2.7
Water[b]				
	0.17	0.01	0.02	–

[a] Treatments 1S and 3S provided Zn, Cu, Mn and Co in sulfate forms. Treatment 1C contained all complexed mineral (Availa-4®; Zinpro Corporation, Eden Prairie, MN). Treatments 2SC and 3SCC contained both complexed and sulfate forms of minerals.

[b] Water was available ad libitum that contained additional trace mineral and was high in sodium (499 ppm) and sulfur (23.3 ppm).

Beginning d 0 through d 100, supplements were measured and individually fed daily in 0.45 kg of wheat middlings before providing bulls with their basal diet. The basal diet of 75.5% corn silage, 10.5% corn, 7% alfalfa hay, and 7% protein supplement was fed to all bulls and was formulated to achieve 1.2 kg ADG. Basal diet nutrient composition was analyzed and is reported in Table 1. The mineral analysis of the basal diet was analyzed by Animal Health Diagnostic Laboratory (Michigan State University, East Lansing MI) using coupled argon-atomic emission spectroscopy (Braselton et al., 1997). The results are reported in Table 2.

Bulls were assigned to one of ten pens with all feed provided in Calan gates and one animal per treatment per pen following an initial collection (-14 d) of semen, blood, scrotal circumference, and body weights. Bulls were allowed a 14 d acclimation period to adapt to individual feeding gates and automatic waterers. Two bulls (both treatment 1C) died during treatment (both unrelated to treatment). Thus, two pens contained only four bulls.

Water was supplied free choice in automatic waterers from a common source. Water was analyzed for mineral content at the Animal Health Diagnostic Laboratory (Michigan State University, East Lansing MI) using coupled plasma-atomic emission spectroscopy (Braselton et al., 1997). Sodium concentration was above recommended

levels, however all other minerals were within acceptable ranges (Table 2).

2.2. Liver biopsies

Liver biopsies were collected on d −21 and 100 utilizing the Tru-cut® needle biopsy technique described by Corah and Arthington (1994). Liver samples were analyzed for Cu, Zn, Mn, and Co at the Animal Health Diagnostic Laboratory (Michigan State University, East Lansing MI) using coupled plasma atomic emission spectroscopy techniques (Braselton et al., 1997). From this analysis, all bulls were considered deficient in Cu and all adequate in Zn (Mertz, 1986) on d −21, thus bulls were not blocked by liver trace mineral status.

2.3. Semen evaluation and scrotal circumference

Semen, scrotal circumference and body weights were collected on d −14, 14, 42, 70, and 98. The same individuals performed scrotal measures, semen collections, and semen analyses over the entire study to eliminate variation. Scrotal circumference was measured at the widest circumference with a manual metal tape (Hammerstedt, 1996). Semen samples were collected by electro-ejaculation and bulls failing to provide an ejaculate were allowed to rest for a short period of time and then another collection was attempted. If bulls did not provide an ejaculate (n = 3 instances out of 246 attempts), semen parameters were recorded missing data. Ejaculate volume was recorded and 10 μl of raw semen was evaluated microscopically at 100x magnification for gross swirl. Progressive motility and strength of motility (rate of movement across field of view) of diluted semen (1:5, v:v in phosphate-buffered saline, pH 7.4) were recorded at 400x magnification on a warmed microscope slide. A sample of raw semen was mixed with an Eosin/Nigrosin morphology stain (Lane Manufacturing Inc., Denver CO.) for morphology and viability evaluation (Lunstra and Echternkamp, 1982). Spermatozoa morphology was evaluated by a counting 100 random cells at 1000x magnification and classifying them as normal or having head or tail abnormalities as described by Barth and Oko (1989). Tail abnormalities and classifications included proximal and distal cytoplasmic droplets, coiled and bent tails, plus any miscellaneous abnormalities. Head abnormalities included abnormal acrosomes and all types of abnormally shaped heads. Concentration of spermatozoa in each ejaculate was determined using a hemocytometer. Puberty was defined as the first collection date at which an ejaculate contained a minimum of 50×10^6 total spermatozoa with at least 10% progressive motility (Lunstra et al., 1978).

On collection d 42, 1.1 ml of semen from each bull was stored at −20 °C for trace mineral analyses. Samples were analyzed at the Animal Health Diagnostic Laboratory (Michigan State University, East Lansing MI) using coupled argon plasma emission spectroscopy techniques (Stowe et al., 1985).

2.4. Blood collection and serum assays

Blood samples were collected on the same days described for semen collections above. Samples were collected in vacutainer tubes (10 ml) via caudal venipuncture at 0, 30, and 60 min following an i.m. injection of GnRH (0.22 μg/kg BW; Fertagyl, Merck Animal Health, Madison, NJ) to measure LH and testosterone concentrations in blood as an indicator of testes maturation (Tannen and Convey, 1977; Fabry et al., 1983). Blood was allowed to clot and stored at 4° C overnight. Serum was harvested by centrifugation at 3000g for 20 min and stored at −20° C until hormone analysis. Serum concentration of testosterone was determined by radioimmunoassay (RIA) using a validated testosterone assay (Siemens Medical Solutions, Los Angeles, CA). Briefly, 50 μl of serum and 500 μl of I^{125} labeled testosterone were incubated in antibody-testosterone coated tubes. Bovine serum standards and serum quality controls containing high and low concentrations of testosterone were included in all assays. Intra- and inter-assay coefficients of variation were 2 and 12%, respectively, and assay sensitivity for testosterone was 0.04 ng/mL.

The LH assays were conducted as described by Niswender et al. (1969). Assay sensitivity was 0.5 ng/mL and the intra- and inter-assay coefficients of variation were 9.6 and 11.9%, respectively. The FSH assay was validated and conducted in Dr. Jon Wheaton (University of Minnesota) according to the NIDDK procedure for radioimmunoassay of FSH (Meyer et al., 1991). Assay sensitivity was 0.3 ng/mL and the intra-assay coefficient of variation was 4.8%.

2.5. Statistical analyses

Data was analyzed as a randomized complete block design using initial pubertal status, age, and scrotal circumference as blocks, and bull as the experimental unit. Differences in spermatozoa morphology and concentration, scrotal circumference, Zn and Cu semen concentrations, age, and number of days to reach puberty were analyzed using the General Linear Model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC, version 9.3). Comparisons of semen and scrotal measures included all bulls and data from each collection date, except that concentration of mineral in semen was only conducted at d 42. Age and number of days on treatment to reach puberty included only those bulls (n = 45) that were not pubertal at trial onset. Differences in concentration of liver mineral levels and serum LH, FSH, and testosterone were analyzed for all bulls and all collection dates using repeated measures using the GLM procedure of SAS (1994). Comparison of puberty status for bulls among treatments was analyzed using categorical analysis (CATMOD procedure) of SAS (1994). All differences were considered significant at $P < 0.10$. For all data, bulls were grouped for two separate comparisons; first by the individual treatment and then by pre-planned comparisons of mineral form and level (sulfate vs complexed; sulfate 1x vs complexed 1x; sulfate 3x vs complexed 3x; 1x vs 2x vs 3x NRC recommended).

Table 3
Mean (±SEM) liver zinc (Zn), copper (Cu) and manganese (Mn) concentrations from peripubertal bulls on d −21 and 100 of mineral supplementation: 1x sulfate form (1S), 1x complexed form (1C), 1S + 1C (2SC), 1S + 2 × 1C (3SCC), and 3 × 1S (3S). Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co.

	d −21 Zn	d 100 Zn	d −21Cu	d 100 Cu	d −21 Mn	d 100 Mn
Mineral supplement						
1S	190.0 ± 8.8	117.7 ± 7.2	18.2 ± 3.9	174.7 ± 7.2	6.4 ± 0.3	6.2 ± 0.3
1C	170.4 ± 11.9	130.6 ± 7.2	22.3 ± 6.3	167.6 ± 18.9	5.9 ± 0.4	6.1 ± 0.2
2SC	201.1 ± 15.3	122.2 ± 6.1	20.7 ± 4.0	214.6 ± 17.9	5.5 ± 0.5	6.6 ± 0.3
3SCC	183.3 ± 16.5	129.8 ± 7.1	21.8 ± 4.1	243.0 ± 22.4	5.5 ± 0.6	6.6 ± 0.4
3S	196.3 ± 13.5	129.9 ± 5.0	22.6 ± 4.3	240.1 ± 20.6	6.3 ± 0.3	6.5 ± 0.2
Treatment		P = 0.59		P = 0.07		P = 0.38
Organic 1x vs inorganic 1x[a]		P = 0.28		P = 0.83		P = 0.99
1x vs 2x vs 3x[b]		P = 0.51		P = 0.01		P = 0.16
Organic vs inorganic[c]		P = 0.60		P = 0.86		P = 0.27
Organic 3x vs inorganic 3x[a]		P = 0.91		P = 0.88		P = 0.33

[a] Comparison of d 100 liver biopsy with d −21 liver biopsy for 1C vs 1S or 3C vs 3S.
[b] Comparison of d 100 liver biopsy with d −21 liver biopsy for 1C and 1S vs 2SC vs 3SCC and 3S.
[c] Comparison of d 100 liver biopsy with d −21 liver biopsy for 1C and 2SC and 3SCC vs 1S and 3S.

3. Results

3.1. Liver mineral concentrations

Liver Cu concentrations of all bulls were well below the minimum adequate level of 100 ppm (Puls, 1995) at the initiation of treatment (Table 3). After 100 d of treatment, liver Cu was increased ($P < 0.01$) to adequate concentrations (100–125 ppm; Puls, 1995; Kincaid, 2000) compared to initial concentrations, in all bulls regardless of treatment (Table 3). Liver copper concentrations of bulls tended to differ ($P = 0.07$) between treatment at the end of the study (Table 3). On d 100, liver Cu concentrations were greater ($P = 0.01$) in 3SCC and 3S bulls compared to 1C and 1S bulls, whereas liver Cu concentrations in 2SC bulls were intermediate.

Initial (d −21) liver Zn concentrations were adequate (> 100 ppm; Puls, 1995) and after 100 d of treatment, all liver Zn concentrations were still within the adequate range (Table 3). Following 100 d of treatment, liver Zn concentrations were not different ($P = 0.59$) between treatments but were decreased ($P < 0.01$) across all treatments. No differences related to mineral form or level were detected ($P > 0.28$).

Initial liver concentrations of Mn were deficient (< 7 ppm; Kincaid, 2000) in all bulls on d −21. Liver concentrations of Mn on d 100 were still deficient in all bulls and not different than d −21 concentrations, nor were they affected by mineral treatment or mineral supplementation form or level ($P > 0.16$; Table 3).

3.2. Puberty

Five bulls (one from each treatment) were pubertal at the initiation of the trial and were removed from the puberty data set. Two bulls did not reach puberty by the end of the supplementation period and were assigned a puberty date of 126 d (or 28 d beyond the end of the evaluation period; Table 4). Mineral treatment had no effect ($P = 0.42$) on mean age at puberty (data not shown) nor mean days on treatment ($P = 0.35$) to reach puberty (Fig. 1). There was a trend for treatment to affect ($P = 0.12$) the percentage

Table 4
Cumulative number of pubertal bulls in each treatment by collection day.

	Collection day					
Treatment[a]	d −14	d 14	d 42	d 70	d 98	Not pubertal
1S	1	3	6	8	9	1
1C	1	3	6	6	7	1
2SC	1	4	7	9	10	0
3SCC	1	2	9	9	10	0
3S	1	2	4	7	10	0
Total	5	14	32	39	46	2

[a] Treatments were 1x sulfate form (1S), 1x complexed form (1C), 1S + 1C (2SC), 1S + 2 × 1C (3SCC), and 3 × 1S (3S). Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co.

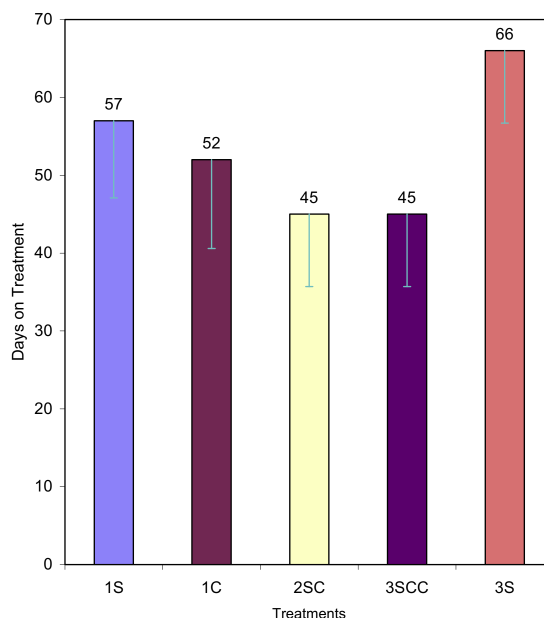

Fig. 1. Mean (-SD) days on mineral treatment for bulls receiving one of 5 mineral supplements to reach puberty ($P = 0.35$). Mineral treatments were; 1x sulfate form (1S), 1x complexed form (1C), 1S + 1C (2SC), 1S + 2 × 1C (3SCC), and 3 × 1S (3S). Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn and 12.5 mg Co.

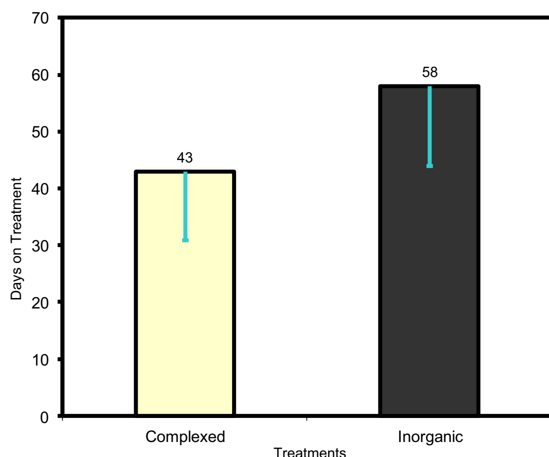

Fig. 2. Number of days on treatment to puberty ($P = 0.11$, mean − SD) for bulls receiving one of two forms of mineral supplement. Bulls are grouped by form of mineral supplements: Complexed [1x complexed form (1C), 1S + 1C (2SC), and 1S + 2 × 1C (3SCC)] and Inorganic [1x sulfate form (1S), and 3 × 1S (3S)]. Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn, and 12.5 mg Co.

of bulls reaching puberty on d 42 as more ($P < 0.05$) bulls receiving 3SCC (90%) were pubertal compared to other treatments (≤ 70%). Bulls fed complexed trace minerals (1C, 2SC, 3SCC) tended ($P = 0.11$) to reach puberty after fewer days on treatment (43.9 ± 5.7 d) than bulls fed only sulfate minerals (58.5 ± 6.7 d; 1S, 3S; Fig. 2). After 42 d of mineral supplementation, more ($P = 0.03$) bulls fed complexed trace mineral (79%) were pubertal compared to those fed only sulfate trace mineral (47%).

3.3. Semen evaluations

No differences in head or tail abnormalities were detected ($P \geq 0.40$) in spermatozoa from bulls receiving different levels or forms of mineral supplement on d −14, 14, 70, or 98 of the study. On d 42, 3S bulls had a greater ($P = 0.08$; 39.2%) percentage of cytoplasmic droplets on spermatozoa compared to all other treatments (1S, 1C, 2SC, 3SCC; 15, 20, 25, and 21.6% respectively). No differences were detected in ejaculate concentration or motility between treatments ($P \geq 0.44$) on any of the semen collection days. No treatment differences ($P \geq 0.10$) were detected in semen concentrations of Zn or Cu on d 42 (only day measured; Fig. 3). Neither semen Zn nor Cu concentration differed ($P \geq 0.10$) when bulls were grouped by date of puberty or comparison of pubertal status (Zn; 1.7 vs 1.3 ppm; Cu; 0.17 vs 0.16 ppm for pubertal and non-pubertal bulls, respectively).

3.4. Scrotal circumference

Scrotal circumference did not differ between bulls receiving different mineral treatments ($P \geq 0.28$) levels ($P \geq 0.14$) or forms ($P \geq 0.11$) throughout the trial. Scrotal circumference increased similarly across bulls in all treatments from d −14 to 100 of the study.

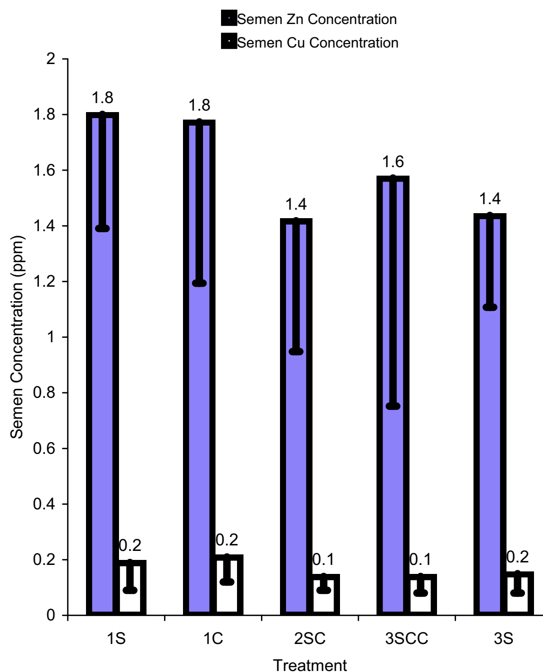

Fig. 3. Zinc and Cu concentration (mean − SD) in semen of bulls ($P > 0.10$) on d 42 of a 100 d mineral supplement study. Mineral supplements were 1x sulfate form (1S), 1x complexed form (1C), 1S + 1C (2SC), 1S + 2 × 1C (3SCC), and 3 × 1S (3S). Each 1x supplementation level contained 360 mg Zn, 125 mg Cu, 200 mg Mn, and 12.5 mg Co.

3.5. Hormone assays

Mean serum concentrations of testosterone, FSH and LH did not differ ($P \geq 0.12$) for bulls receiving different mineral levels or forms. However, mean testosterone concentrations were greater ($P < 0.01$) in serum from bulls at puberty than the collection date before reaching puberty across all treatments (Fig. 4). When the prepubertal mean concentrations of LH and FSH in serum collected at the previous collection date were compared to the mean concentration in serum at the pubertal collection date, no difference were detected ($P \geq 0.10$). However, the LH area under the curve was decreased ($P < 0.01$) in the pubertal blood sample (563 ± 29 ng/ml, Mean ± SEM) compared to the previous pre-pubertal sample (706 ± 43 ng/ml). Serum LH and testosterone concentrations were different ($P < 0.01$) between the 0, 30, and 60 min time intervals after the GnRH injection. Mean (± SEM) serum LH were 1.08 ± 0.02, 13.42 ± 0.16, and 9.62 ± 0.10 ng/ml and testosterone were 4.08 ± 0.12, 6.05 ± 0.13, and 10.08 ± 0.14 ng/ml for the time 0, 30, and 60 min blood samples, respectively. Mean serum LH, testosterone and FSH concentrations were different ($P < 0.01$) between collection dates. Mean (± SEM) serum LH on d −14, 14, 42, 70, and 98 were 9.25 ± 0.54, 7.36 ± 0.40, 8.72 ± 0.50, 7.78 ± 0.43, and 7.11 ± 0.39 ng/ml, respectively. Mean (± SEM) serum testosterone on d −14, 14, 42, 70, and 98 were 4.39 ± 0.25, 4.76 ± 0.21, 7.78 ± 0.30, 8.71 ± 0.24, and 8.04 ± 0.25, respectively. Mean (± SEM) serum FSH on d −14, 14, 42, 70, and 98 were 0.88 ± 0.02,

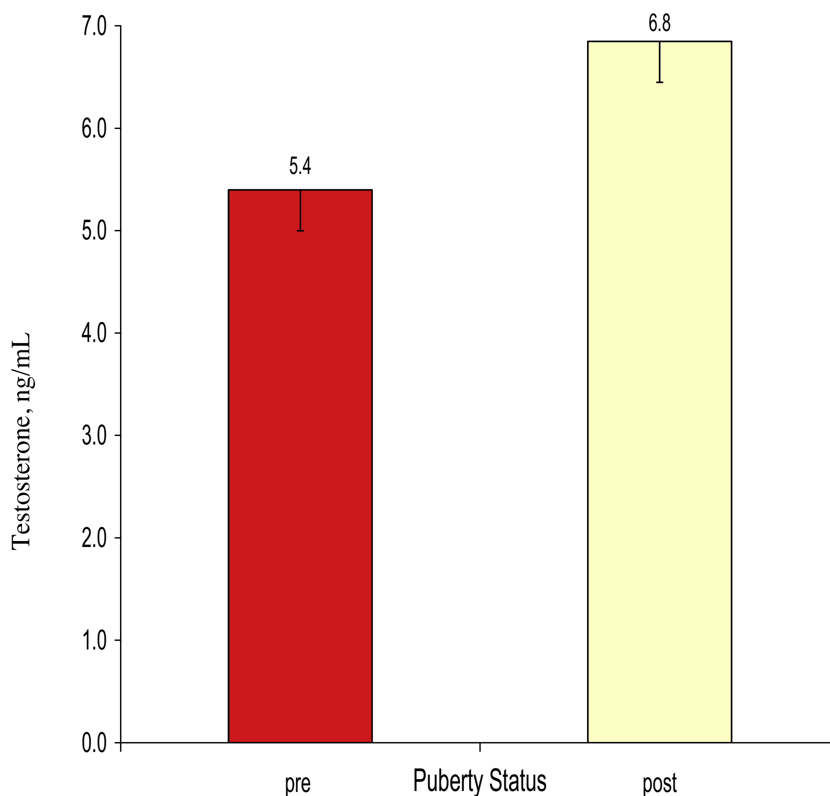

Fig. 4. Serum testosterone concentration ($P < 0.01$, mean, SD) of bulls on the collection day before puberty (pre) compared to the collection day in which they were considered pubertal (post).

0.90 ± 0.02, 0.93 ± 0.02, 0.88 ± 0.02, and 0.96 ± 0.02 ng/ml, respectively.

4. Discussion

Many beef bulls fail initial breeding soundness exams when evaluated at or less than one year of age. This evaluation is usually conducted on yearling bulls prior to purebred herd production sales. Purebred producers are at a disadvantage when these bulls fail and increased pressure is placed on accelerating puberty and sexual maturity to increase sperm production. Thus, the value of this research is to evaluate ways that might accelerate pubertal onset and sexual maturation in young bulls. Also, puberty is difficult to define and measure in the bull and is a gradual process rather than an event. Onset of puberty can be defined behaviorally, when spermatozoa first appear in the ejaculate or urine, or when the ejaculate contains a threshold number of spermatozoa that are likely capable of reaching the site of fertilization (Lunstra et al., 1978). Frequent collection of ejaculates from bulls are difficult and could even alter the age at which puberty is defined due to depletion of epididymal reserves. However, because age of puberty differs between bulls, repeated assessments are needed to estimate age of puberty. We realize that 28 d evaluation of ejaculates provides only an approximate date of pubertal onset, but more frequent assessment is not very realistic.

Very little information is available in the literature regarding the role of trace minerals on reproductive health or on puberty establishment in bulls. Normal liver Zn concentrations (DM) ranged from 83 to 300 ppm (mean, 111 ppm) for aged cows (Mertz, 1986). Trace mineral supplements were removed from bulls in the present study 81days before treatment, yet liver Zn concentrations were all above the adequate liver Zn concentrations (100 ppm) as proposed by Mertz (1986). The range forage at this location is typically well below adequate concentrations of Zn at this location (Grings et al., 1996), so it was surprising to find adequate liver Zn concentrations in weaned bull calves without supplementation. It is theorized that the body has very strong homeostatic mechanisms involved in Zn absorption and metabolism (Kendall et al., 2000). Thus, when rations are deficient in Zn, the body may become more efficient in maintaining and recycling Zn stores within the body. As an example, many intestinal brush border carrier membrane proteins are influenced by nutritional and physiological status and these proteins quickly increase in number during Zn depletion in the diet (Cousins, 1985) allowing for more Zn to be absorbed by the mediated diffusion mechanism. Increased absorption of Zn during a fast (Mertz, 1986) and increased storage during late gestation of cows (Graham, 1991; Swenson, 1999; Vierboom et al., 2003) has been reported. Swenson (1999) suggested that in preparation for high requirements of Zn during parturition and lactation, the body absorbed and

stored Zn more efficiently during late gestation. The initial Zn liver concentrations of bull calves in this study following 81 d of no mineral supplementation (Table 3) suggest that the bull calves may have been able to compensate for the lack of Zn supplementation by lowering Zn excretion and increasing Zn recycling.

Liver Zn concentrations of all bulls except one, on d 100 of treatment were decreased relative to d −21 (Table 3), suggesting an increased use of Zn during the peripubertal stage. Even the 20 bulls fed the high levels of minerals (either complexed or inorganic forms) had decreased ($P < 0.01$) liver Zn content on d 100 relative to d −21. Semen Zn concentration on d 42 (Fig. 3) appears to be negatively associated with interval to puberty. It seems plausible that as bulls reach puberty and continue to mature, they will have a higher Zn concentration in their ejaculates due to the higher concentration of mature spermatozoa. This concept is supported at least, in the testis, by Bedwal and Bahuguna (1994) who reported Zn content to be higher in the adult testis compared to the immature testis. Pitts et al. (1966) reported decreased testis growth in Zn deficient bulls at two to five months of age, but the bulls in our study were not deficient (based on pre and post-study liver Zn concentration) and no effect on testis growth (scrotal circumference) was realized. Although no previous studies have investigated Zn liver concentrations of bulls during this age period, Swenson (1999) reported that liver Zn concentrations decreased in heifers during their first lactation regardless of form or level of Zn supplementation. Parizek et al. (1966) reported that testis Zn concentrations decreased to normal adult rat concentration levels after a peripubertal increase. Crichton et al. (1982) reported that low Zn intake by young males of several species interfered with normal sexual development. Several authors (Parizek et al., 1966; Mason et al., 1982; Salem et al., 1984; Mertz, 1986) have reported altered testis growth and only immature spermatozoa among male rats that were deficient in Zn. It would have been interesting to have continued this study to determine if continued sexual maturity would further decrease Zn liver concentrations in these bulls consuming summer forages that are generally deficient in Zn and whether any differences in fertility might have been revealed.

In the current study, sperm measures were collected at 28 d intervals to measure changes in semen traits and assign approximate pubertal dates. The danger that comes from such assessment is that 28 d between pubertal assment is a fairly long period, but weekly observations in young bulls might not be appropriate either because more frequent collections could alter the age at which puberty is defined due to depletion of epididymal reserves. Thus, some caution is warranted in assigning absolute age of puberty values in studies such as this. More bulls receiving the complexed mineral supplement (1C, 2SC, 3SCC) were pubertal at 42 d compared to bulls receiving sulfate mineral supplements (1S and 3S). The trend for more bulls receiving the 3SCC treatment (as compared to all other treatments) to be pubertal on d 42, suggests additional complexed mineral may decrease age of puberty in bulls regardless of trace mineral status. Initially, the 42 d pubertal response to supplement appeared to have occurred too early to be related to treatment, as the total spermatogenic cycle in the bull takes approximately 61 d (Amann, 1970). However, much of the influence Zn has on spermatogenesis occurs during the later stages. Parizek et al. (1966) reported a rapid increase in the testis Zn concentration (120 to 200 μg/g at 35 and 58 d of age, respectively) in rats during the stage of sexual development that coincides with the first completion of spermiogenesis (first spermatids transforming into spermatozoa). Elevated testis Zn concentration in rats during this stage of development was thought to be due to the high content of Zn bound to protein in the mature sperm cell. Additionally, testosterone contributes a larger role in the later stages of spermatogenesis, by quantitatively maintaining meiosis and spermiogenesis (Courot and Ortaveant, 1981). During spermiogenesis, there is an increase in the activity of lactate dehydrogenase, a Zn dependent enzyme used in the mitochondrial sheath of the spermatozoa for ATP production in anaerobic conditions (Parizek et al., 1966). Development of spermatids into spermatozoa in the bull requires approximately 17.2 d with transportation through the epididymis requiring approximately 14 d (Amann and Almquist, 1962) which makes the 42-d response to supplementation more reasonable. Arthington et al. (2002) and Rowe et al. (2014) also reported improvements in bull fertility when Zn proteinate or complexed trace minerals compared to Zn sulfate or sulfate minerals were supplemented.

Because liver Zn concentrations were not below adequate levels at the start or end of the study, perhaps Cu was the more limiting mineral affecting sexual maturity in bulls in this study. Very little is known about the specific role, if any, Cu plays in the male reproductive system. Reduced libido and spermatogenesis have been attributed to a Mo antagonist induced Cu deficiency in bulls (Thomas and Moss, 1951). Deb et al. (2014) reported that Cu deficiency may delay onset of puberty, but no literature directly linking Cu to specific processes in bovine male reproduction has been reported. In the current study, the role of Cu in establishment of puberty is unclear due to the lack of adequate controls. However, bulls were deficient in liver Cu concentrations at the initiation of supplementation and all bulls demonstrated an increase in liver Cu concentrations by the end of supplementation (Table 3). Thus, inadequate Cu was not an obvious factor in any traits measured and any effects of Cu on fertility measurements in the present study cannot be separated from pubertal advancement. The Mn levels in liver are normally quite low and didn't change in bulls in this study. There was no effect of treatments on liver Mn concentration after 100 days of supplementation. This agrees with Masters et al. (1988) and indicates that the liver may not be a primary storage organ for manganese.

This is the first study to demonstrate a possible role of mineral form on age of puberty in bulls. Further investigation into this area, may improve our understanding of the effect mineral level and form may have on days to puberty. Such an effect could be significant to the bull industry, as increased production demands are expected of bulls. In addition, various authors have reported spermatozoa quantity and quality continue to improve after puberty (Almquist and Amann, 1975; Almquist et al., 1976; Lunstra and Echternkamp, 1982; Evans et al., 1994) thus,

earlier attainment of puberty would likely increase lifetime sperm production and increase odds of passing a breeding soundness exam early in life.

Another aspect deserving attention is the possible negative effect a high sulfate treatment may have on the pubertal process of bulls. Statistical comparison of select individual treatments is not valid when a "treatment effect" was not realized. However the variation in days on treatment to puberty leads to concerns that a negative effect may exist. There was a 21 d variation in days to puberty between bulls fed elevated sulfate only minerals (3S) compared to bulls fed a similar amount of complexed mineral (3SCC; Fig. 1). Swenson (1999) reported a similar negative response in first calf heifers, where more heifers fed either complexed mineral or no mineral were pregnant to their first service (AI) compared to heifers fed sulfate trace minerals. Smart et al. (1985) reported that a diet with 0.3% sulfates decreased plasma Cu yet had no effect on plasma Zn. The presence of high sulfur reduced the bioavailability of selenium and interfered with copper metabolism (Spears, 2003), but any effect high sulfur might have on Zn utilization was not reported. Further investigations may be warranted to study the effects high sulfates may have on bull reproduction.

Mineral level or form in the current study did not affect spermatozoa motility, concentration, head or tail abnormalities. The increased percentage of cytoplasmic droplets on d 42 among 3S bulls was not surprising because at 42 d, the 3S treatment group had the fewest number of pubertal bulls. Lunstra and Echternkamp (1982) reported a rapid decrease in ($P<0.01$) the percentage of spermatozoa with proximal cytoplasmic droplets in bulls that reached puberty. Barth and Oko (1989) stated that in many cases, an ejaculate with a high percentage of cytoplasmic droplets indicates lack of maturity within the male reproductive system. Kendall et al. (2000) reported increased spermatozoa viability in rams that received a bolus containing 15.2% Zn, 0.5% Co, and 0.15% Se compared to rams that received no bolus.

Due to the variation in the quality of ejaculates obtained in the current study and the difficulties in obtaining ejaculates repeatedly, it was difficult to truly assess differences in traits such as spermatozoa numbers, motility, and morphology. Also, even though bulls in the current study reached puberty, they may not have reached full reproductive capacity (Abdel-Raouf, 1960). Additional stress imposed on bulls in the current study caused by several trips through the chute on collection days may have affected our ability to obtain a representative sample from every bull. Some bulls that had reached puberty failed to ejaculate during subsequent collection attempts. This presented a major problem in the current study and thus, prevented extensive evaluation of spermatozoa characteristics. The use of an artificial vagina may have allowed for collection of a more representative sample.

Scrotal circumference was also measured and analyzed to monitor reproductive response to mineral supplementation. There are conflicting results on scrotal circumference and Zn deficiencies in the literature. Several authors have reported inadequate scrotal growth when bulls or rams were severely deficient in Zn (Pitts et al., 1966; Underwood and Somers, 1969; Martin and White, 1992). The bulls in the current study were fed at least the NRC recommended levels and were not deficient in Zn at the beginning of treatment, while bulls in the above studies were fed little or no Zn. Arthington et al. (2002) reported no change in scrotal circumference between yearling bulls supplemented with levels at or above NRC (2000) recommendations. Because bulls in the current study were fed adequate Zn levels, under NRC (2000) standards, it is not surprising that scrotal growth was unaffected.

The increase in serum testosterone and decrease in LH area under the curve during the hour following GnRH administration in pubertal bulls is likely the result of stimulation of LH release followed by a negative feedback effect of testosterone on LH (Tannen and Convey, 1977). We had some concern that GnRH administration each month might hasten onset of puberty, and is possible that it did, but because it was performed on all bulls, it would not have been expected to affect potential treatment responses. Whether testosterone or LH response following GnRH administration could serve as an endocrine measure of puberty or not deserves further study.

In summary, copper and zinc availability varied with age and physiological state of peripubertal bulls and are influenced by diet and supplementation level and type. Liver concentrations of copper were suppressed in bulls post weaning while liver concentrations of zinc appeared adequate. Addition of sulfate or complexed forms of these minerals increased liver concentrations of copper, but liver concentrations of zinc decreased in bulls, likely due to increased zinc utilization in the pubertal bull. We demonstrate that zinc utilization by the pubertal bull is high and suggest the National Research Council recommendations be increased for peripubertal bulls. After 100 d of trace mineral supplementation, liver Zn concentrations were lower than d −21 even when Zn was supplemented at 3x recommended levels. There was a trend for complexed mineral to decrease the number of days to puberty, thus use of at least some complexed mineral in diets of peripubertal bulls may be beneficial. No differences existed in semen characteristics or scrotal measurements of bulls supplemented with different mineral forms. Serum concentrations of LH, FSH, and testosterone were measured in bulls following administration of GnRH to assess changes related to pubertal status. Testosterone concentrations were increased and LH area under the curve decreased in pubertal bulls compared to prepubertal bulls and further research is warranted to determine if a threshold response can be indicative of pubertal status.

Conflict of interest

None of the authors nor institutions they represent have any conflicts of interest.

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